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EpiQuik™ DNA MethyltransferaseActivity/Inhibition Assay Kit

Base Catalog # P-3001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ DNA MethyltransferaseActivity/Inhibition Assay Kit is suitable for non-radioactively measuring DNMT activity or inhibition from a broad range of species including mammals, plants, and bacteria.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	48 Assays Cat. #P-3001-1	96 Assays Cat. #P-3001-2	Storage Upon Receipt
M1 (10X Wash Buffer)	11 ml	22 ml	4°C
M2 (DNMT Assay Buffer)	1.5 ml	3 ml	4°C
M3 (Adomet, 8 mM)*	35 µl	70 µl	–20°C
M4 (DNMT Positive Control)*	5 µl	10 µl	–20°C
M5 (Capture Antibody)*	5 µl	8 µl	4°C
M6 (Detection Antibody, 200 µg/ml)*	10 µl	20 µl	–20°C
M7 (Developer Solution)	6 ml	12 ml	4°C
M8 (Stop Solution)	3 ml	6 ml	4°C
8-Well Assay Strips (With Frame)	6	12	4°C
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** For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts, one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt, store **M3**, **M4**, and **M6** at –20°C away from light; store **all other components** at 4°C away from light.

***Note**: Check if wash buffer, **M1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.*

The kit is stable for up to 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Microplate reader with the ability to read at 450 nm
- ☐ Orbital shaker
- ☐ Pipette and pipette tips
- ☐ 1.5 ml microcentrifuge tubes

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

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Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Usage Limitation: The EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

Epigenetic inactivation of genes plays a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. Four active DNMTs have been identified in mammals – DNMT1, DNMT2, DNMT3A, and DNMT3B. The inhibition of DNMTs may lead to demethylation and expression of the silenced genes. DNMT inhibitors are currently being developed as potential anticancer agents.

Currently, there are very narrow selections of methods to use for measuring DNMT activity or inhibition. These methods are time consuming, labor-intensive, have low throughput, or produce radioactive waste. Epigentek addresses these problems with the EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit by using a unique procedure to measure DNMT activity or inhibition.

The EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit has the following advantages and features:

- Extremely fast procedure, which can be completed within 3 hours.
- Innovative colorimetric assay without radioactivity, extraction, or chromatography.
- Strip microplate format makes the assay flexible, allowing manual or high throughput analysis.
- Simple, reliable, and consistent modification conditions

PRINCIPLE & PROCEDURE

The EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit is designed for measuring total DNMT activity (de novo, maintenance) via a non-radioactive 96-well plate format. In an assay with this kit, the unique cytosine-rich DNA substrate is stably coated on the strip wells. These wells are specifically treated to have a high DNA absorption ability. DNMT enzymes transfer a methyl group to cytosine from Adomet to methylate the DNA substrate. The methylated DNA can be recognized with a 5-methylcytosine antibody. The ratio or amount of methylated DNA, which is proportional to enzyme activity, can then be colorimetrically quantified through an ELISA-like reaction.

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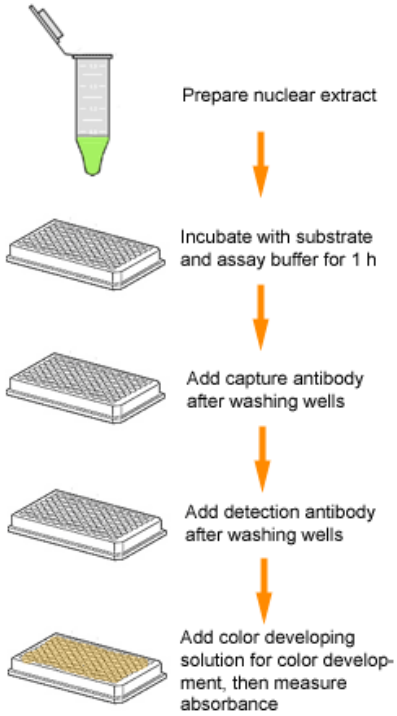
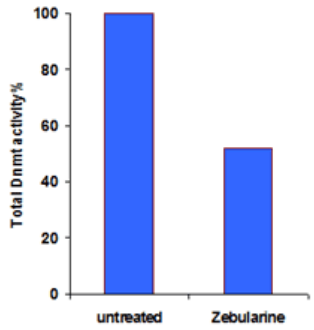
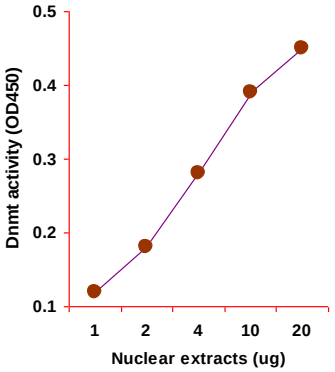

Prepare nuclear extract
Incubate with substrate and assay buffer for 1 h
Add capture antibody after washing wells
Add detection antibody after washing wells
Add color developing solution for color development, then measure absorbance

Schematic procedure of the EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit.



HSC-3 cells were incubated with/without zebularine (220 uM) for 48 h. Nuclear proteins were extracted and total Dnmt activity was measured.



Nuclear extracts were prepared from MCF-7 cells using EpiQuik™ Nuclear Extraction Kit and total Dnmt activity was measured.

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

1. Prepare nuclear extracts by using your own successful method. For your convenience and the best results, Epigentek offers the *EpiQuik™ Nuclear Extraction Kit* (Cat. No. OP-0002-1) optimized for use with this product. Nuclear extracts can be used immediately or stored at –80°C for future use.

2.

a. Predetermine the number of strip wells required for your experiment. Remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Predetermine your sample wells, positive control wells, DNMT inhibition wells, and blank wells.

c. Dilute **M1**, which is 10X concentration, with distilled water (pH 7.2-7.5) into 1X concentration (**1X M1**).

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3.

a. Dilute **M3** with **M2** (at a 1:5 ratio) to 1.6 mM.

b. Add 21 µl of **M2** to each of your DNMT inhibition wells. Add 24 µl of **M2** to each of the other wells.

c. Add 3 µl of the **Diluted M3** to each well.

d. Sample: add 3 µl of your nuclear extracts (4 to 20 µg) or purified DNMT enzymes to the sample wells.

e. Positive Control: add 0.5 to 1 µl of **M4** and 2 to 2.5 µl of **M2** to each of the positive control wells.

f. DNMT Inhibition: add 3 µl of tested inhibitors to each of the DNMT inhibition wells at different concentrations.

g. Blank: add 3 µl of **M2** to each of the blank wells.

h. Mix and cover all wells with Parafilm M and incubate at 37°C for 1.5 hours.

4.

Aspirate and wash each well with 150 µl of **1X M1** 3 times.

5.

a. Dilute the **M5** (at a 1:1000 ratio) to 1 µg/ml with **1X M1**.

b. Add 50 µl of **Diluted M5** to each well and incubate at room temperature for 60 min on an orbital shaker (50 to 100 rpm).

6.

Aspirate and wash each well 4 times with 150 µl of **1X M1** each time. This can be done by simply pipetting **1X M1** in and out of the wells.

7.

a. Dilute **M6** (at a 1:1000 ratio) to 0.2 µg/ml with **1X M1**.

b. Add 50 µl of **Diluted M6** to each well and incubate at room temperature for 30 min.

8.

Aspirate and wash each well with 150 µl of **1X M1** 5 times.

9.

Add 100 µl of **M7** into each of the wells and incubate at room temperature for 2-10 min away from light. Begin monitoring color development in the sample and the positive control well for a medium blue color.

10.

Add 50 µl of **M8** to each well to stop enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color will change to yellow and the absorbance should be read on a microplate reader at 450 nm within 2 to 15 min

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Calculate DNMT activity or inhibition using the following formulas:

$$DNMT\ Activity\ (OD/h/mg) = \frac{No\ Inhibitor\ OD - Blank\ OD}{Protein\ Amount\ (\mu g)^{*} \times hour^{**}} \times 1000$$

* Protein amount added into the reaction at Step 3d.
** Incubation time used at Step 3h.

$$DNMT\ Inhibition\ \% = \left(1 - \frac{Inhibitor\ Sample\ OD - Blank\ OD}{No\ Inhibitor\ Sample\ OD - Blank\ OD}\right) \times 100\%$$

TROUBLESHOOTING

Problem	Possible Causes	Suggestions
No signal in both the positive control and the sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order and if some steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
No signal or weak signal in only the positive control wells	The **M4** positive control enzyme is insufficiently added to the well in Step 3e.	Ensure sufficient amount of **M4** positive control enzyme is added in Step 3e.
	The positive control enzyme has lost activity due to improper storage.	Follow the Shipping & Storage guidance in this User Guide for storage of positive control.
No signal in only the sample well	The protein sample is not properly extracted.	Ensure the nuclear protein extraction protocol is suitable for DNMT protein extraction. Sodium chloride concentration of the extraction buffer should not be more than 100 mM. Alternatively, use the EpiQuik™ Nuclear Extraction Kit (Cat. No. OP-0002-1).
	Insufficient protein amount added to well.	Ensure extract contains a sufficient amount of protein.
	The sample is not prepared from fresh cells or tissues.	The nuclear extracts obtained frozen cells or tissues significantly lose enzyme activity. Fresh samples should be used.

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	Nuclear extracts are improperly stored or have been stored for an exceedingly long period.	Ensure the nuclear extracts are stored at -80°C for no greater than 6 weeks.
	Absence of DNMT activity in the sample due to treatment.	N/A.
High background in blank	Insufficient washing of wells.	Check if washing recommendations at each step is performed according to the protocol.
	Contaminated by the positive control.	Ensure the well is not contaminated from adding enzyme accidentally or from using enzyme contaminated tips.
	Over-development.	Decrease the color development time (incubation time in Step 9) before adding **M8** Stop Solution in Step 10.

RELATED PRODUCTS

Sample Preparation

OP-0002	EpiQuik™ Nuclear Extraction Kit
OP-0022	EpiQuik™ Nuclear Extraction Kit II (Nucleic Acid-Free)
OP-0003	EpiQuik™ Whole Cell Extraction Kit

DNA Methyltransferase Assay

P-3004	EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit (Fluorometric)
P-3006	EpiQuik™ DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Kit
P-3007	EpiQuik™ DNA Methyltransferase 3B Activity/Inhibitor Screening Assay Kit
P-3011	EpiQuik™ DNMT1 Assay Kit
P-3012	EpiQuik™ DNMT3A Assay Kit
P-3013	EpiQuik™ DNMT3B Assay Kit
P-3021	EpiQuik™ MBD2 Binding Activity/Inhibition Assay Kit

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